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Epidemiological Analysis of the Schisis Association in the Spanish Registry of Congenital Malformations

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Since its description by Czeizel [1981: Am J Med Genet 10:25–35], there has been general acceptance of the schisis association as a distinct entity although, to the best of our knowledge, no other epidemiological study has confirmed its existence. Here we present an epidemiologic study on schisis defects and their associations with each other in children with and without blastogenetic defects. This study demonstrates that most cases represent the dysmorphogenetic response of the primary developmental field. Am. J. Med. Genet. 70:16–23, 1997.

KEY WORDS: developmental field defects; blastogenesis; associations; schisis association; epidemiology

INTRODUCTION

Since its description by Czeizel [1981], the schisis association has been universally recognized as a distinct entity although, to the best of our knowledge, no subsequent epidemiologic study has confirmed its existence.

Here we present an epidemiologic study of schisis defects and their associations with each other in children with and without blastogenetic defects. This study demonstrates that most cases represent the dysmorphogenetic response of the primary developmental field.

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MATERIALS AND METHODS

The study was based on the 22,264 live and stillborn malformed infants identified by the Spanish Collaborative Study of Congenital Malformations (ECEMC). This is a hospital-based case-control study and surveillance system. All children born in about 62 participating hospitals from all over Spain are examined by physicians who, interested in the problem of congenital defects, collaborate with the ECEMC program and follow its unique and strict methodology. Children are examined during the first 3 days of life to identify major and/or minor/mild defects. For each case, the next nonmalformed infant of the same sex born in the same hospital is selected as a control subject. Once the case and control infants have been identified, the same physicians interview the mothers of case and control infants to gather information on family history, obstetrical data, and prenatal exposures. In many instances, photographs, karyotypes, imaging studies, pathology reports, and results of other studies are also available for review. Detailed descriptions of the ECEMC methodology have been published elsewhere [Martínez-Frías, 1995; Martínez-Frías et al., 1991, 1995; Martínez-Frías and Urioste, 1994].

Between April 1976–March 1994, the ECEMC controlled a total population of 1,135,177 liveborn infants and 7,536 stillborn between January 1980–March 1994. Among them, 21,835 liveborn and 429 stillborn infants were selected as cases because they had major and/or mild defects and some minor anomalies detected during the first 3 days of life.

For the purposes of this investigation, we selected the same schisis defects studied by Czeizel [1981]: neural tube defects (anencephaly (AN), encephalocele (EN), and spina bifida (SB)), omphalocele (OM), diaphragmatic defects (diaphragmatic hernia or agenesis of diaphragm (DH)), cleft lip ± cleft palate (CL), and cleft palate (CP). To test if the associations among these defects were due to the fact that all are midline defects, we analyzed, in malformed male infants, the association of each schisis defect with hypospadias (HY), a nonschisis, nonblastogenetic midline anomaly.

To study the preferential association of different schisis defects, we used the adjusted ratio between observed (O) and expected (E) values, by applying the method proposed by Khoury et al. [1990], which controls the nonspecific tendency of defects to cluster among themselves. The analysis was done separately on infants with blastogenetic defects [Opitz, 1993], on those without blastogenetic defects, and on the sum of these two groups. We have considered as blastogenetic malformations all those defects that could be produced or induced during the first 4 weeks of gestation (Table I).

TABLE I. Blastogenetic Defects Included in Present Study

Conjoined twins	Esophageal duplication
Acardia-acephalus twinning	Agenesis of the stomach
Otocephaly	Intestinal duplication
Atelencephaly-aprosencephaly	Agenesis of the gallbladder
Anencephaly	Accessory gallbladder
Encephalocele	Renal agenesis
Spinda bifida	Agenesis of the adrenal glands
Holoprosencephaly	Bladder duplication
Anophthalmia	Exstrophy of bladder
Anotia	Exstrophy of cloaca
Amelia	Anal atresia
Anomalies of the body stalk/wall	Sirenomelia
Limb-body wall complexes	Caudal regression
Ectopia cordis	Sacrococcygeal teratoma
Pentalogy of Cantrell	Acrorenal field defect
Conotruncal septation defects	Polyasplenia field defect
DiGeorge sequence	Situs inversus
Diaphragmatic defects	Axial mesodermal dysplasia complex
Tracheal agenesis	Vertebral segmentation anomalies
Lung agenesis	Spondylocostal/thoracic dysostosis
Tracheoesophageal fistula	

RESULTS

Table II separates the study population of malformed infants into those with and without schisis defects, and shows that 11.03% (2,455) of cases had at least one of the studied schisis-type of defects. Among them, 72.87% (1,789) were infants with only schisis defects, while the remaining 27.13% (666) had schisis defects plus other defects. Table III shows the distribution of infants with and without blastogenetic defects by the presence or absence of any schisis defect. Among the 22,264 malformed infants, blastogenetic defects were present in 2,322 (10.43%) cases. Of these, 53.83% (1,250) had at least one of the selected schisis defects, 39.02% (906) had only schisis defects, and 14.81% (344) had schisis defects plus other defects. These figures contrast with those observed among the 19,942 malformed infants without blastogenetic defects, in whom only 6.04% (1,205) had at least one of the schisis defects ($P \ll 0.0001$), 4.43% (883) with only schisis defects and 1.61% (322) with schisis defects plus other abnormalities. The data in Table IV indicate that midline defects were present, as expected, in 100% of children with schisis defects, but only in 22.81% of children without schisis defects ($P \ll 0.0001$). In addition, the proportion of midline defects among infants with blastogenetic defects (86.85%) was significantly higher than in those without blastogenetic defects (19.14%) ($P \ll 0.0001$).

Table V shows the distribution of the 2,455 infants with schisis defects with or without other defects (Table II) by number of schisis-type defects and by the presence or absence of blastogenetic defects. Among the total, two or more schisis defects were observed in 79 (6.32%) of the children with blastogenetic defects, but in only 7 (0.58%) children without blastogenetic defects ($P \ll 0.0001$). Analysis of infants with blastogenetic defects showed that two or more schisis defects were present in 2.21% (20/906) of infants with only schisis defects, in contrast with 17.15% (59/344) of those with schisis plus other defects ($P \ll 0.0001$).

Table VI presents the different combinations of two or more schisis defects observed among the different groups of malformed infants. To analyze each type of association, we included all types of combinations, without taking into consideration if some could be part of the same anomaly (sequence), such as AN + CP, AN + SB, and EN + SB. This is a way to confirm that they are in fact the same anomaly. Table VII shows the number of infants with each one of the different schisis-type defects in our total sample as well as in males, separated by clinical presentation in children with and without blastogenetic defects.

The results of the analysis of the observed associations using the adjusted ratio between observed (O) and expected (E) values, applying the method of Khoury et al. [1990], are presented in Table VIII. The analysis was done using the total number of cases with at least one of the schisis defects in children with and without blastogenetic defects and in the total number of malformed infants. The results of the analysis on the total number of cases are essentially concordant with

TABLE II. Study Population: Proportion of Malformed Children With and Without Schisis Defects

All malformed infants	With only schisis defects	With schisis plus other defects	Without schisis defects	Total	
				N	%
With schisis defects, N (%)	1,789 (72.87)	666 (27.13)		2,455 (100)	11.03
Without schisis defects, N			19,809	19,809	88.97
Total, N (%)	1,789 (8.04)	666 (2.99)	19,809 (88.97)	22,264 (100)	100

TABLE III. Study Population: Proportion of Malformed Children With and Without Schisis Defects Among Infants With and Without Blastogenetic Anomalies

All malformed infants	With schisis defects: With only schisis defects	With schisis defects: With schisis plus other defects	With schisis defects: Total	Without schisis defects	Total: N	Total: %
With blastogenetic defects, N (%)	906 (39.02)	344 (14.81)	1,250 (53.83)	1,072 (46.17)	2,322 (100)	10.43
Without blastogenetic defects, N (%)	883 (4.43)	322 (1.61)	1,205 (6.04)	18,737 (93.96)	19,942 (100)	89.57
Total, N (%)	1,789 (8.04)	666 (2.99)	2,455 (11.03)	19,809 (88.97)	22,264 (100)	100

those observed by Czeizel [1981], although some combinations of schisis defects, such as CP + OM, CP + DH, SB + CL, SB + CP, SB + DH, are not statistically associated. However, when we analyzed the different associations among children with blastogenetic defects, we observed that most of the associations between the blastogenetic schisis defects occurred with a frequency that was not higher than their nonspecific tendency to cluster with other *blastogenetic* defects. There is only one exception: the observed number of cases of SB + DH was significantly lower than expected. On the other hand, it is of interest that while anencephaly is preferentially associated with cleft lip and cleft palate, spina bifida is not.

Table IX presents the total number of cases with hypospadias and their combination with each schisis-type defect, and Table X depicts the results of the adjusted O/E analysis. Although HY + CL showed a significant association at a level of $P < 0.04$ in children with some blastogenetic defects, one of the cases also had SB. If we exclude this case, the association between HY + CL is not statistically significant. Thus, it appears that only SB is preferentially associated with HY, as we described previously [Martínez-Frías, 1994].

Table XI shows the number of cases with affected first-degree relatives for each schisis defects, indicating the type of schisis anomaly observed in the index patient and in their affected relative(s).

DISCUSSION

Czeizel [1981] observed 130 (0.29%) out of 44,608 malformed infants, who had two or more (of what he called) schisis defects without other major congenital malformations. In our study we identified (Table V) 20 (0.09%) out of 22,264 cases with two or more schisis defects not known to have other major or minor defects. The difference is statistically very significant ($P \ll 0.0002$). On the other hand, the proportion of cases with two or more schisis-type defects with other malformations was 0.18% (80/44,608) in the study by Czeizel [1981], and 0.30% (66/22,264) in ours ($P < 0.002$). However, the proportion of cases with two or more schisis defects, with or without other defects, among the total cases was 0.47% (210/44,608) in the Hungarian program [Czeizel, 1981] and 0.39% (86/22,264) in ours, a difference that is not statistically significant ($P = 0.12$). Nevertheless, we analyzed all types of combinations, and so if we exclude from Table VI those cases having AN + EN and AN + SB, as well as those with EN + SB, considered by Czeizel [1981] as having only AN and EN, respectively, the percentages observed in our data are significantly lower: 0.04% for cases with only schisis defects and 0.29% for total cases. However, the percentage among cases with schisis plus other defects (0.26%) is still significantly higher in our data ($P < 0.0006$) than in those of Czeizel [1981] (0.18%). Consequently, it appears that, since Czeizel considered only major defects, the inclusion of minor/mild defects in our study may be responsible for the higher frequency of cases with other congenital defects, and the lower frequency of cases with only schisis defects we observed. In fact, if we consider only the total of 2,455 cases with at least one schisis anomaly (Table V), the proportion of cases with two or more schisis defects is higher among infants with schisis plus other major or minor/mild defects (2.69%) than among those with only schisis defects (0.81%).

The frequency of different types of combinations among schisis defects (Table VI) also differs from Czeizel's findings. The most frequent combination he observed was AN + CL (33 cases out of 130), while the most frequent associations in our analysis (Table VI) were CL + OM and OM + DH (with 8 cases out of 86, respectively), if we do not take into account SB + EN (9 cases out of 86), because Czeizel considered these cases as having only encephalocele. OM + DH, one of the two most frequent combinations in our data (8 cases of 86), was observed only three times in Czeizel's material. However, Czeizel excluded syndromal cases and other multiple congenital anomaly (MCA) patterns, while we considered all cases. The number of cases with three or

TABLE IV. Distribution of Malformed Children With Midline Defects in Relation With the Presence or Absence of Schisis Defects Among Infants With and Without Blastogenetic Anomalies

Malformed infants	Midline defects in children with at least one schisis defect	Midline defects in children without schisis defects	Total
With blastogenetic defects, N (%)	1,250/1,250 (100)	931/1,072 (86.85)	2,322
Without blastogenetic defects, N (%)	1,205/1,205 (100)	3,587/18,737 (19.14)	19,942
Total, N (%)	2,455/2,455 (100)	4,518/19,809 (22.81)	22,264

TABLE V. Children With Schisis Defects: Distribution by Number of Schisis Anomalies Among Infants With and Without Blastogenetic Defects

	Only schisis defects			Schisis plus other defects			Total	
Malformed infants	One	Two or more	Total	One	Two or more	Total	Two or more	Total
With blastogenetic defects, N (%)	886 (70.88)	20 (1.6)	906 (72.48)	285 (22.8)	59 (4.71)	344 (27.52)	79 (6.32)	1,250 (100)
Without blastogenetic defects, N (%)	883 (73.28)	0 (–)	883 (73.28)	315 (26.14)	7 (0.58)	322 (26.72)	7 (0.58)	1,205 (100)
Total, N (%)	1,769 (72.06)	20 (0.81)	1,789 (72.87)	600 (24.44)	66 (2.69)	666 (27.13)	86 (3.50)	2.455 (100)

more schisis defects was higher in Czeizel's data. We do not know if voluntary interruption of gestations (VIG) could account for these differences as well as for the overall frequency of infants with two or more schisis-type defects, since Czeizel did not comment on whether or not VIG was possible in Hungary at the time of his study. VIG has been legal in Spain since 1986, and we think it may definitely affect the frequency at birth of these cases. In our program this could be particularly true for cases with three or more schisis defects, as well as for neural tube defects (NTDs) and for OM, two defects that have shown a significant decreasing tendency in their secular frequency in our data since 1986 [Bermejo et al., 1994]. Nevertheless, we cannot totally exclude the possibility that other environmental or genetic factors could also account for the observed differences between our results and those of Czeizel [1981].

In Table II we showed that 11.03% of malformed infants in our sample had schisis defects. This proportion, however, was 10.67% (2,329/21,835) for liveborn and 50.82% (218/429) for stillborn infants. Similarly, in Table III we can see that 10.43% of all malformed infants had at least one blastogenetic defect, and that this proportion varied from 9.48% (2,071/21,835) among liveborn to 58.51% (251/429) among stillborn infants. The higher frequency of blastogenetic defects among stillborn infants strongly supports the contention that blastogenetic defects are highly lethal, as suggested by Opitz [1993] and epidemiologically demonstrated by us [Martínez-Frías, 1995]. On the other hand, as previously documented [Martínez-Frías, 1995], most children with blastogenetic defects have midline defects, while the proportion is significantly lower in infants without blastogenetic defects (Table IV).

We analyzed the preferential association of schisis defects on the total number of malformed infants, using the method proposed by Khoury et al. [1990]. In addition, we also separated the cases between those with and without blastogenetic defects (Table VIII), since two of the four schisis defects studied are of blastogenetic origin (NTD and DH), while the other two may

TABLE VI. Combinations of Schisis-Type Anomalies in the Same Child

	Among children with at least one blastogenetic defect		Among children without blastogenetic defects		
Schisis-type combinations	Only Schisis defects (N = 1,250)	Schisis plus other defects (N = 1,072)[a]	Only schisis defects (N = 1,205)	Schisis plus other defects (N = 18,737)[a]	Total (N = 22,264)
AN + SB	2	3	0	0	5
AN + CL	2	3	0	0	5
AN + CP	3	4	0	0	7
AN + DH	2	1	0	0	3
EN + CL	0	2	0	0	2
EN + CP	0	7	0	0	7
EN + OM	0	1	0	0	1
EN + DH	1	0	0	0	1
SB + EN	7	2	0	0	9
SB + CL	0	3	0	0	3
SB + CP	0	2	0	0	2
SB + OM	0	7	0	0	7
SB + DH	1	2	0	0	3
CL + OM	0	3	0	5	8
CL + DH	0	7	0	0	7
CP + OM	0	0	0	2	2
CP + DH	0	3	0	0	3
OM + DH	2	6	0	0	8
AN + SB + CL	0	1	0	0	1
EN + SB + OM	0	1	0	0	1
EN + CL + OM	0	1	0	0	1
Total	20	59	0	7	86

[a]Syndromes are included.

TABLE VII. Total Number of Children and Male Infants With Each of the Schisis Anomalies by Clinical Presentation*

	Children with at least one blastogenetic defect		Children without blastogenetic defects		Total	
	Male (N = 1,184)	Total (N = 2,322)	Male (N = 10,458)	Total (N = 19,942)	Male (11,642)	Total (N = 22,264)
Anencephaly						
Isolated	143	290	0	0	143	290
MCA	14	31	0	0	14	31
Total	157	321	0	0	157	321
Spina bifida						
Isolated	175	375	0	0	175	375
MCA	50	109	0	0	50	109
Total	225	484	0	0	225	484
Encephalocele						
Isolated	30	61	0	0	30	61
MCA	20	56	0	0	20	56
Total	50	117	0	0	50	117
Cleft lip						
Isolated	0	0	307	467	307	467
MCA	28	54	61	112	89	166
Total	28	54	368	579	396	633
Cleft palate						
Isolated	0	0	135	373	135	373
MCA	20	49	72	139	92	188
Total	20	49	207	512	227	561
Diaphragmatic hernia						
Isolated	106	174	0	0	106	174
MCA	39	86	0	0	39	86
Total	145	260	0	0	145	260
Omphalocele						
Isolated	0	0	41	70	41	70
MCA	14	35	30	48	44	83
Total	14	35	71	118	85	153

*MCA, multiple congenital anomalies, including syndromes. This includes cases with other defects apart from the one that is studied in each line.

not be (oral clefts and OM). The analysis of associations among the total population of malformed infants showed essentially the same results observed by Czeizel [1981]. That is, most of the schisis defects tend to cluster among themselves more frequently than the nonspecific tendency of defects to cluster. However, for the following schisis defects combinations, SB + CP, SB + CL, SB + DH, CP + OM, and CP + DH, the observed number of cases did not differ from the number expected and, thus, these combinations were not statis-

TABLE VIII. Schisis-Type Combinations: Adjusted O/E (Method of Khoury et al., 1990)

Schisis association	Children with at least one blastogenetic defect (N = 2,322)		Children without blastogenetic defects (N = 19,942)		Total (N = 22,264)	
	O/E	$P <$	O/E	$P <$	O/E	$P <$
AN + SB	0.54	NS*			5.17	0.001
AN + DH	0.43	NS			4.05	0.01
EN + OM	2.94	NS			7.5	0.001
EN + DH	0.41	NS			3.92	0.04
SB + EN	1.59	NS			15.15	0.001
SB + DH	0.14	0.001			2.06	NS
SB + OM	4.19	0.001			8.33	0.001
OM + DH	5.33	0.001			11.49	0.001
AN + CL	3.82	0.001			3.28	0.001
AN + CP	5.22	0.001			3.61	0.001
EN + CL	1.76	NS			3.09	0.04
EN + CP	4.79	0.001			6.80	0.001
SB + CL	1.20	NS			1.20	NS
SB + CP	0.67	NS			0.58	NS
CL + OM	5.13	0.001	6.58	0.001	1.23	0.001
CL + DH	2.73	0.01			3.44	0.001
CP + OM	1.79	NS	2.25	NS	1.38	NS
CP + DH	1.28	NS			1.39	NS

*NS, not significant.

TABLE IX. Total Number of Infants With Hypospadias and Each One of the Schisis Anomalies by Clinical Presentation

	Children with at least one blastogenetic defect	Children without blastogenetic defects	Total
Hypospadias (HY)			
Isolated	0	1,873	1,873
MCA	42	151	193
Total	42	2,024	2,066
HY + AN	2	0	2
HY + SB	14	0	14
HY + EN	0	0	0
HY + CL	3	9	12
HY + CP	1	7	8
HY + OM	1	2	3
HY + DH	2	0	2

tically associated. Nonetheless, we cannot totally exclude that these combinations could be affected by VIG.

It is noteworthy, on the other hand, that the observed numbers of most of the combinations of schisis defects of *blastogenetic* origin did not differ from the expected numbers in relation with other *blastogenetic* defects (first column of Table VIII). In other words, the tendency for schisis defects of blastogenetic origin to cluster among themselves is not greater than their tendency to cluster with other *blastogenetic* defects. This result suggests that all of these blastogenetic schisis defects are the response of "damage" to the primary field and, as such, *the frequency of the association between two schisis defects of this primary field should be similar (and not statistically different from) to the clustering with other defects of this field.* As shown in Table VIII, there is only one exception: SB + DH, for which the observed number of cases is significantly lower than the expected one. However, this result may be biased by VIG, since a fetus with NTD + DH may be more susceptible to VIG than one with only NTD or only DH. *Thus, the combinations of schisis-type defects that are not statistically significant among the group of blastogenetic defects should be considered different dysmorphogenetic manifestations of damage to the primary developmental field.* This necessarily implies that these combinations of schisis defects must be etiologically heterogeneous. In fact, in a review of our data, we observed combinations of at least two of some of the schisis-type defects in trisomy 13 and 18, Meckel syndrome, Fryns syndrome, and Wiedemann-Beckwith syndrome, and in children of diabetic mothers as well as in sporadic cases of infants with MCA patterns. This epidemiologic information strengthens the postulate that the combination of blastogenetic schisis-type defects constitutes the response of the primary developmental field, and consequently is preferentially associated.

Most of the statistically significant associations observed in Table VIII among children with at least one blastogenetic defect (first column) are those in which one of the schisis defects of blastogenetic origin was statistically associated with other schisis defects that we considered of nonblastogenetic origin. In all but two, the nonblastogenetic defect was CL or CP. In the remaining two, it was OM associated with SB, in one case, and with DH, in the other. It is of interest that oral clefts preferentially associate with AN but not with SB. This observation supports the assumption that cleft palate (and also cleft lip?) observed in children with AN could be a consequence of the alteration of craniofacial structures constituting a sequence and, consequently, representing a nonrandom tendency to be associated more frequently with AN than with other defects of the primary field. The same explanation could account for the association of OM + DH, in which DH may alter the morphogenetic movements of the intestines and lead to OM. However, it is more difficult to relate SB sequentially to an alteration of morphogenetic movement producing OM. Thus, these two defects may be related through their causal factors. CL was preferentially associated with OM in the three study groups: in children with blastogenetic defects, in those without blastogenetic defects, and in the total number of cases. This supports the assumption that, in cases without chromosomal or Mendelian syndromes, this is a preferential (schisis) association of two defects that although in most cases appear to be of organogenetic origin, may in fact be defects of blastogenesis.

The fact that a blastogenetic defect such as SB is significantly associated with an apparent nonblastogenetic schisis defect (OM) may suggest that, at least in some cases with normal chromosomes, the nonblastogenetic defects may be a mild expression of an early event or a remnant that has been almost "canalized" back to normal. However, combinations of two or more schisis-type defects among infants with other nonschisis defects, as well as the significant preferential association between schisis defects of blastogenetic origin (such as EN, SB, and DH) and of nonblastogenetic origin (such as OM and CL), may be due to a chromosomal

TABLE X. Combination of Schisis Anomalies and Hypospadias: Adjusted O/E (Method of Khoury et al., 1990)

	Children with at least one blastogenetic defect		Children without blastogenetic defects		Total	
	O/E	*P* <	O/E	P <	O/E	*P* <
AN + HY	1.32	NS*			0.78	NS
SB + HY	6.01	0.001			3.51	0.001
CL + HY	3.16	0.04	1.49	NS	1.56	NS
CP + HY	1.43	NS	1.80	NS	1.64	NS
DH + HY	0.92	NS			0.75	NS
OM + HY	2.04	NS	1.69	NS	1.82	NS

*NS, not significant.

TABLE XI. Recurrence in First-Degree Relatives*

Number of cases	Type of defects in index patient	Affected relatives	
		Relative	Type of schisis defect
Only schisis defects			
11	SB	Sibs	SB
4	AN	Sibs	AN
1	AN	Sibs and aunt	SB
1	SB	Twin	AN + SB + EN
1	DH	Sibs	DH
1	Agenesis of diaphragm	Sibs	Hiatal hernia
1	AN	Sibs	SB
2	SB	Mother	CL
1	AN	Father	CL
8	CL	Mother	CL
13	CL	Father	CL
2	CL	Twins	CL
3	CL	Sibs	CL
1	CL	Sibs	DH
5	CP	Mother	CP
3	CP	Father	CP
4	CP	Sibs	CP
1	CP	Twin	CP
Schisis + other defects			
2	EN	Sibs	EN (Meckel S.)
2	AN	Sibs	AN
2	SB	Twins	SB
1	SB	Sibs	AN
2	CL	Father	CL
3	CL	Sibs	CL
1	CL	Sibs	CL (Bar.-Papas S.)
1	CL	Sibs	Cleft uvula
1	CL	Sibs	SB + CL
1	DH	Sibs	DH
1	DH + CP	Sibs	DH
1	OM	Conjoined twins	OM
1	OM	Sibs	OM
2	CP	Mother	CP
1	CP	Sibs	Cleft uvula
1	CP	Twin	CP

*S., syndrome; Bar., Bartsocas.

abnormality, a single gene defect, or a long-term environmental agent. The high proportion of combinations involving oral clefts further supports this suggestion since, as pointed out by Czeizel [1981] affected sibs were only observed among index patients with the association of NTD and oral clefts. As shown in Table XI, we observed only one infant with the schisis-type combination DH + CP who had one affected sib with DH, and one child with isolated CL who had a sib with SB + CL. The differences between the number of cases with affected first-degree relatives observed by Czeizel [1981] and by us could well be due to differences in the genetic background of the Hungarian and Spanish populations. On the other hand, some of the cases with affected sibs observed by Czeizel [1981] could represent any of the X-linked recessive midline defects described after publication of his report [Toriello and Higgins, 1985; Martínez-Frías, 1994].

It is well-known that midline defects tend to cluster among themselves [Opitz and Gilbert, 1982; Khoury et al., 1989; Mathias et al., 1987; Martínez-Frías, 1994]. Thus, to determine if the association of schisis defects was due to the fact that all of them are midline defects, we analyzed the association, among males, of every schisis defect with HY, a nonschisis, nonblastogenetic midline anomaly. As shown in Table X, only the observed cases with HY + SB were significantly higher than the expected ones, supporting our previous suggestion that this constitutes a preferential association or an X-linked recessive syndrome [Martínez-Frías, 1994]. We do not consider the association of HY + CL a preferential one, first, because of the level of significance, and second, because when we exclude the case that also had SB, the number of observed cases does not differ from the number expected.

In conclusion, our epidemiological study on the so-called schisis association shows that the tendency of schisis defects to associate among themselves is significantly higher than the tendency to associate with other nonschisis defects, as observed by Czeizel [1981]. However, when we separate the cases into those with blastogenetic defects and those without blastogenetic defects, the specific tendency for schisis defects of blastogenetic origin to associate among themselves is not greater than their tendency to be associated *with other blastogenetic defects.* This result strongly suggests that the combination of two schisis defects of *blastogenetic* origin represents the dysmorphogenetic reaction of the primary developmental field. The observation of two or more of these schisis-type defects in individuals with syndromes of different cause (causal heterogeneity) supports this conclusion. The results also suggest that some of the observed statistically significant combinations of two schisis defects, such as AN + CP and OM + DH, may represent blastogenetic sequences.

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